Procedures in Family Practice

Diagnostic Procedures of the Skin

Part Two: Skin Biopsy and other Tests

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The diagnosis of skin lesions involves the same principles and approaches required for other clinical problems. "Shotgun" therapy based on visual recognition alone is not an adequate clinical approach. A pertinent history and careful physical examination, supplemented by carefully selected diagnostic procedures, is usually necessary for skin diseases.

The indications, limitations, interpretation, and techniques of diagnostic procedures must be well understood to obtain reliable and useful information. The selection of the specific method of skin biopsy, for example, may be based on cosmetic considerations, location and nature of the lesion, and the physician's understanding of the biology and histopathology of the suspected diagnosis.

This is the second paper in a two-part series dealing with diagnostic procedures of the skin. Various kinds of skin biopsy, touch imprints, the Tzanck smear, and immunofluorescent studies are described as they relate to the everyday practice of the family physician.

The first paper in this two-part series on diagnostic procedures of the skin described the indications, limitations, and interpretation of the Wood's light examination, the KOH slide examination, Gram's stain, and cultures in everyday office practice. This second and concluding paper will discuss four additional procedures – the skin biopsy, touch imprints, the Tzanck smear and immunofluorescent studies.

Skin Biopsy

Skin biopsy is a very important part of the evaluation of cutaneous diseases. Careful selection of the lesion and expertise in dermatopathologic interpretation are necessary to obtain the greatest measure of reliable and specific information. This requires careful clinical judgment.

The chosen lesion should be representative of the eruption. Excoriations, secondary infection, and irritant changes may so alter the microscopic characteristics that the histopathologic interpretation is made difficult and becomes uncertain. A lesion unaltered by these and other factors is desirable and may have to be obtained from a site which the patient cannot reach.

The age of the lesion may also be critical to the provision of an accurate diagnosis. In some, the evolutionary changes may take a few days to develop both the typical clinical and histopathologic characteristics. Biopsying these too early may present non-specific features. In blistering diseases, however, the reverse is true. The vesicle should be biopsied within 48 hours, preferably within 24 hours, to capture the characteristic changes. This principle applies whether the vesicle is intraepidermal or subepidermal (Figure 1). In the healing process the subepidermal vesicle will progress into the epidermis, confusing the interpretation. Therefore, biopsying early lesions is critical to the diagnosis of blistering diseases.

Various other factors must be taken into consideration in selecting a lesion for biopsy. If lesions are available in cosmetically and non-cosmetically important areas, the latter should obviously be chosen, provided it meets the criteria of representative lesion and shows no secondary changes. Additional factors in the decision would be the ease of wound closure, effect on function of the anatomic part, and potential healing. The lower third of the leg of patients over 40, for example, heals more slowly, especially if the circulation is altered by venous or arterial disease. Selection of other lesions or a lesion on the more proximal part of the leg would be preferred. Lesions over the anterior tibia do not heal as well as other sites on the leg. If the lesion is over the anterior tibia, a narrow excision with as much of the excision line away from the tibial edge as possible is desirable.

Special comment should be made about nodular lesions of the lower leg. Biopsy of erythema nodosum and panniculitis is difficult because the disease involves the fat, producing a thickened, unyielding tissue which is difficult to close. One should make a deep narrow ellipse biopsy with the sides of the incision perpendicular to the surface. These may be closed more easily with little suture tension and they heal more rapidly. For these reasons we avoid punch biopsies of the foot and leg, especially anteriorly and in older patients, and prefer suture closure.

There are many ways to do biopsies of the skin. The knowledgeable physician will vary these to suit the lesion and the site. This requires sound clinical judgment and awareness of the biology and histopathology of the disease under consideration.

The diameter or width of the biopsy is less important than the

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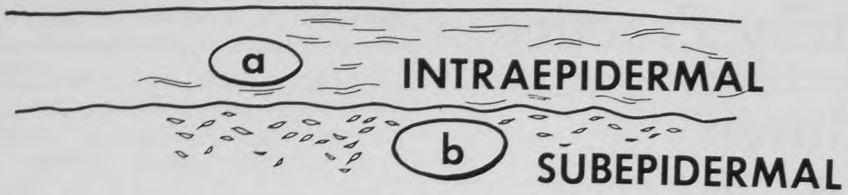

Figure 1. The location of vesicles within the skin is important to the histopathological interpretation. As healing occurs the subepidermal vesicle (b) may become intraepidermal (a). Therefore, a biopsy should be performed on an early vesicle, one no older than 48 hours and preferably less than 24 hours.

depth. As a general rule, the biopsy should be deep enough to include fat. There are exceptions to this dictum which require a degree of dermatologic knowledge. Many of the diseases basically affecting epidermal or dermal changes, such as seborrheic keratoses, warts, molluscum contagiosum, and papulosquamous eruptions, may be shaved or in some instances biopsied with a large skin curette. Basal cell carcinoma presents a special problem if the definitive treatment chosen is curettage and electrodesiccation. Curettage for basal cell carcinomas is accomplished by curetting the softer tumor away from the underlying firm normal dermis. If a preceding biopsy breaks through the dermis into the fat, there is a confusing and false impression as to whether the tumor penetrates into the fat because of the loss of the dermal base. Therefore, there are advantages in doing shave biopsies for basal cell carcinoma.

The basic principles of all minor surgery apply to skin biopsy. General medical status, drug allergies, present medication, and bleeding diathesis should be known. Procaine HCl (Novocain) and chloroprocaine HCl (Nesacaine) may provoke an allergic reaction and cross-react with each other. Lidocaine (Xylocaine) and mepivacaine HCl (Carbocaine) may cross-react with each other but do not cross-react with procaine. If it is unclear whether the patient has an allergy to either or both groups, antihistamines such as diphenhydramine (Benadryl) may be used as a substitute local anesthetic.

Scalpel biopsies may be *excisional* or *incisional* depending on whether the lesion is completely removed or not. For an incisional biopsy the specimen does not have to be wide. The sides of the incision should be parallel to each other and perpendicular to the surface and extending into the fat (Figure 2). Beveling the edges of a narrow biopsy will not permit you to reach the fat (Figure 3). For small biopsies, a Beaver scalpel with a small blade is very useful. The wound is closed with appropriate sutures or adhesive paper strips. Bleeding from a small vessel may be stopped by grasping it with a hemostat or fine forceps and touching the instrument with a spark gap electrosurgery unit (Bovie, etc). The operator should wear gloves to avoid self-injury from the conduction of the current.

Punch biopsies are easily performed and provide satisfactory tissue for examination. The punch should be at least 3 to 4 mm in diameter, but a 2 mm punch, on a rare occasion, carefully obtained and properly imbedded, may be adequate to provide a diagnosis. The smaller punch sizes are especially useful in cosmetically important areas. It should be noted, however, that most pathologists agree that 2 mm of tissue provides too scanty a specimen and there is greater risk of losing it. The punch should be very sharp so that it will easily cut through the skin without much pressure or distortion. Used punches can be resharpened. The large handled punches (Keyes) are not as easily used as those developed for hair transplantation (Orentreich). The latter have small knurled handles which allow easy rotation. It should be emphasized that sharp punches are very important and should cut through tissue easily and without much rotation. As the punch reaches the fat there is a definite give; the specimen will tend to pop up with a slight degree of pressure on the sides. The tissue should be gently held and elevated by one edge so as not to crush or create artifacts in the tissue. A small skin hook or bent needle used for anesthetic injection may be used to elevate the lesion with the least specimen injury. Fat should be attached to the specimen. This requires gentle traction and usually iris scissors to cut across the base in the fat. Hemostasis can almost always be achieved by pressure. Packing with Gelfoam, or very light chemical cautery is occasionally useful. Electrocoagulation at times may be necessary, but this method is not always effective because the bleeders may come from the edge deep in the wound. Tissue injury may also be increased by deep or prolonged electrosurgery.

The advantages of the punch biopsy are its simplicity, rapidity, and the avoidance of suturing in most instances. Some of the potential disadvantages are slower healing, character of the scar, lack of depth in some sites, the possible loss of fat, and difficulty in suturing. To overcome some of these disadvantages, the skin should be stretched perpendicular to the lines of elective surgery or wrinkle lines (Figure 4A). When the skin is released after the biopsy, an oval rather than a round defect remains (Figure 4B). The oval is more easily sutured, may heal more rapidly and may leave a cosmetically more desirable scar after larger punch biopsies. The reservation about adequate depth again necessitates clinical judgment. Lesions in the deep fat and very thick lesions are not suitable for punch biopsies.

Shave biopsies are simple to perform and are very satisfactory and indeed desirable in some circumstances. The *saucerization biopsy* is a variant of shave biopsy with more

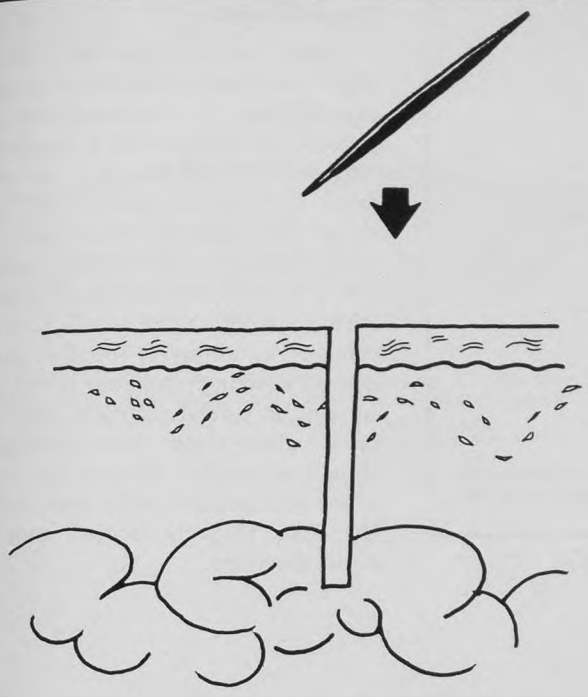

Figure 2. A narrow biopsy has many advantages, especially if performed on the lower leg. But the edges must be parallel to each other and perpendicular to the skin surface in order to reach the fat.

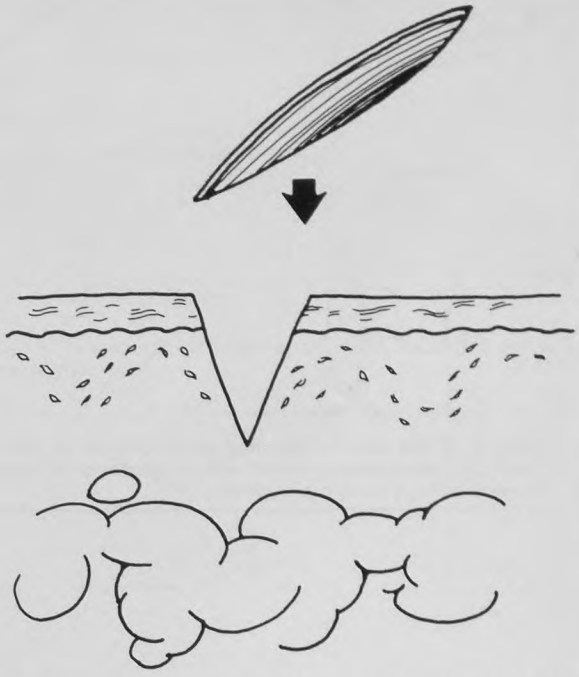

Figure 3. A biopsy, especially a narrow one in which the edges are beveled, may not include the fat. Fat should be included in most skin biopsies. A wide biopsy is more difficult to close.

controlled depth to the procedure. For a simple lesion a scalpel blade transects the lesion at the base tangential to the surrounding surface skin (Figure 5). A small piece of razor blade obtained by breaking a steel blade (stainless steel does not break well) or with the blade breaker-holder instrument will transect the base more easily because of its sharpness and thinness. If more depth is desirable or the lesion is flat, a biopsy can be carried out by a number of techniques, all with similar end results. The anesthetic may elevate the lesion so that a shave can be done (Figure 6), or the skin gently squeezed between the thumb and index finger to permit shaving across the protruding skin. Another method inclines the scalpel in the direction of the lesion and an incision encircles approximately one half of the biopsy (Figure 7A). The free edge of the skin is then grasped with forceps or retracted with skin hooks and the specimen excised to a desirable depth (Figure 7B). Hemostasis is achieved in a manner similar to that used in the punch biopsy technique. All shave and saucerization methods have special value if fat is not sought in the specimen (see section on basal cell carcinoma). However, if the nature of the lesion is unclear, or if greater depth is needed, or if the clinician is not sufficiently familiar with the disease process, then biopsy methods which include fat are preferred.

Biopsy with a curette is another simple way to sample a lesion and possibly remove it. The fragments may be more difficult to interpret by the pathologist, but it is highly satisfactory with certain lesions. Perhaps its most important use is for seborrheic keratoses, which are very superficially placed in the skin. The lesion can be sprayed with Frigiderm both for local anesthesia and to produce a firm surface. The smaller lesion can be removed with a rapid, firm but not heavy pull of the curette in the direction of the physician. The scrapings should be sent for histopathologic examination. Hemostasis can be achieved by a pressure dressing. Molluscum contagiosum can also be treated in this way. A deeper curettage will remove the softer, mushy part of a basal cell carcinoma and may be submitted for diagnosis. This method may also provide definitive treatment. Usually it is preferable to obtain a tissue report before undertaking definitive treatment requiring extensive procedures.

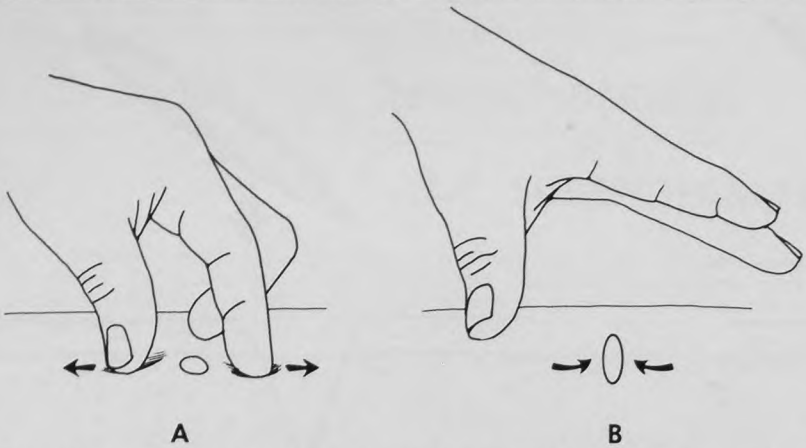

Figure 4. If the skin is stretched perpendicular to the skin lines for a round punch biopsy (A), the resultant defect will be an ellipse in the direction of the skin lines (B). The oval lesion is more easily closed.

Touch Imprints

Touch imprints provide a method which may add information to tissue examination. If a cutaneous lesion is suspect of representing a lymphoma, then touch imprints may be made from the biopsy material to allow better visualization and study of individual cells. The biopsy tissue is gently blotted once against a dry gauze to remove the excess blood. Then the side of the biopsy is touched gently, but with enough pressure to leave an imprint at several different sites on a clean glass slide. The pathologist should be notified that you have made a touch-imprinted slide, and include the slide, properly labeled, with the tissue specimen.

Tzanck Smear

A Tzanck smear is a rapid test used in the diagnosis of bullous diseases. It is most helpful in the diagnosis of viral infections and more difficult but useful in pemphigus vulgaris. From a vesicle no more than 48 hours old or preferably 24 hours old or less, the blister top is removed and the base of the lesion is gently scraped. This material is applied to a clean slide and allowed to dry. We stain our slide in the following manner. The slide is air-dried. Undiluted Geimsa stain is next applied to the slide for 45 seconds, then rinsed in tap water, and subsequently dried. The slide is covered by a cover slip and examined for mononuclear and multinuclear giant cells of viral infection and acantholytic cells of pemphigus.

Table 1. Immunofluorescence – Direct and Indirect

	Direct	Indirect
Pemphigus	+ Intercellular (epidermal)	+
Bullous pemphigoid	+ Tubular band at the basement membrane	+ about 50%
Benign mucosal pemphigoid	+	+ only a few reported as positive
Erythema multiforme	–	–
Dermatitis herpetiformis	+ to IgA (only occasionally to IgG as the others). Location in basement membrane zone and papillary dermis adjacent to lesion and occasionally in the lesion.	–
Systemic lupus erythematosus	+* Granular or thready or homogeneous band in basement membrane area.	
Cutaneous lupus erythematosus (discoid LE)	+ In lesion similar in location and appearance to SLE. None seen in normal skin of patient.	

*Positive in the patient's normal appearing skin, especially light protected; renal involvement more likely and valuable in the diagnosis of systemic lupus erythematosus.[4,5]

Immunofluorescent Studies

The antinuclear antibody test (ANA, antinuclear factor test, ANF) has contributed to the understanding of the diagnosis and prognosis of

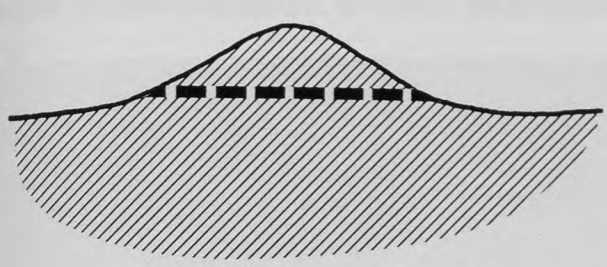

Figure 5. For many lesions a transection of the elevated portion is a very simple and satisfactory method of biopsy. The dotted line represents the direction of the incision.

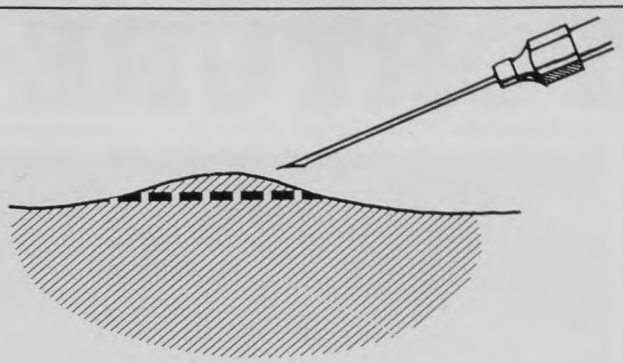

Figure 6. The anesthesia may elevate a flat lesion permitting a shave biopsy.

certain skin diseases.[1-3,6] Most important, immunofluorescent studies of the skin have been adding new information to the study of bullous eruptions.[3,6]

Certain fluorochrome dyes, when exposed to ultraviolet light, produce a fluorescence which can be seen with the fluorescent microscope. If this dye is attached to an antibody to human serum globulin, then fluorescence will be observed at sites where this antibody binds with the globulin. The immunofluorescent studies are referred to as either *direct* or *indirect.* In the indirect immunofluorescence technique the patient's *serum* is incubated with a normal substrate which may include normal skin and monkey esophagus, and tissue specific circulating antibodies will be identified. Direct immunofluorescence requires the study of the patient's *skin* (from a lesion or a normal site). The results of immunofluorescent studies are summarized in Table 1. In lupus erythematosus the possibility of renal involvement may be suspected if immunofluorescence is present in clinically normal, sun-protected skin.[3-5] In brief, immunofluorescence may be very helpful in the diagnosis of bullous disease and lupus erythematosus. But because the tests are not always positive (particularly with the indirect method), clinical judgment and interpretation are just as important as in other laboratory studies.

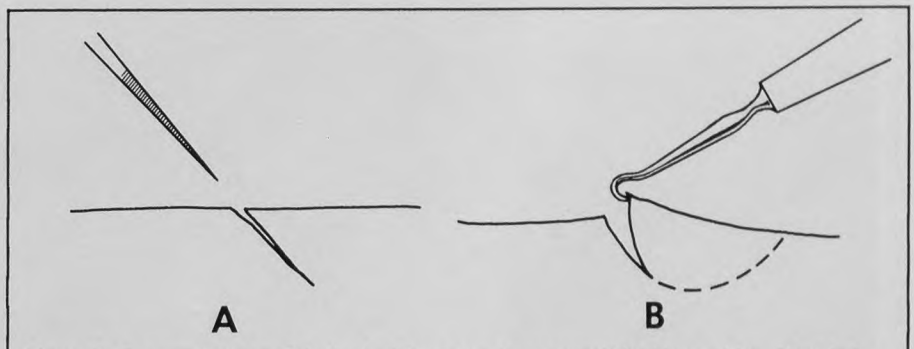

Figure 7. For most types of lesions a biopsy can be accomplished by making a beveled incision in the direction of the lesion (A) and then retracting the lesion while completing the undercutting at the appropriate depth (B).

Comment

In the diagnosis of skin lesions the physician should develop a clinical approach similar to that used for other medical problems and not depend on visual recognition alone. Some characteristics of skin lesions – morphology, configuration, and body location – have been stressed. The technique, indications, and limitations of selected diagnostic procedures have been described. Most important, knowledgeable clinical judgment is required in the selection, performance, and interpretation of these tests.

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